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Supplemental Information

Newly Identified Nematodes from Mono Lake Exhibit Extreme Arsenic Resistance

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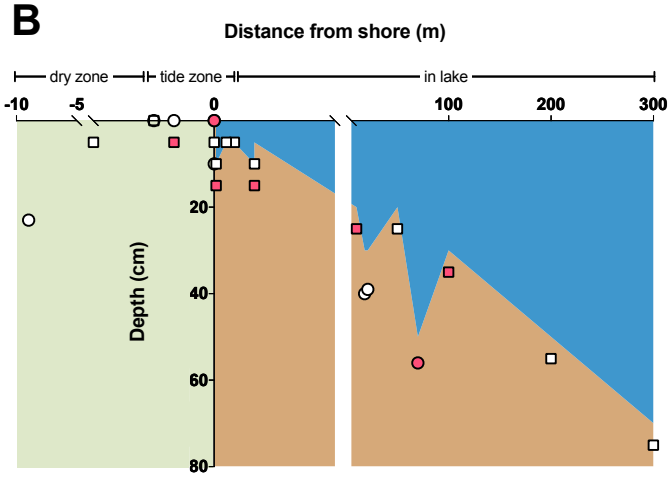
A

"Pristine Beach"
Secluded

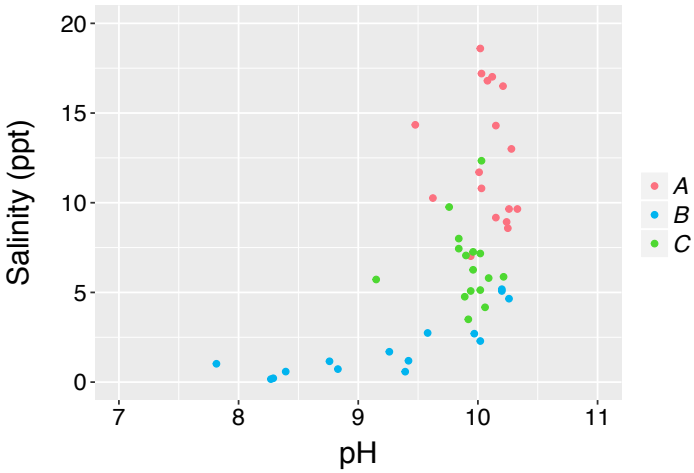
"Navy Beach"
Tufa-rich

"Old Marina"
Rocky shore

B



C

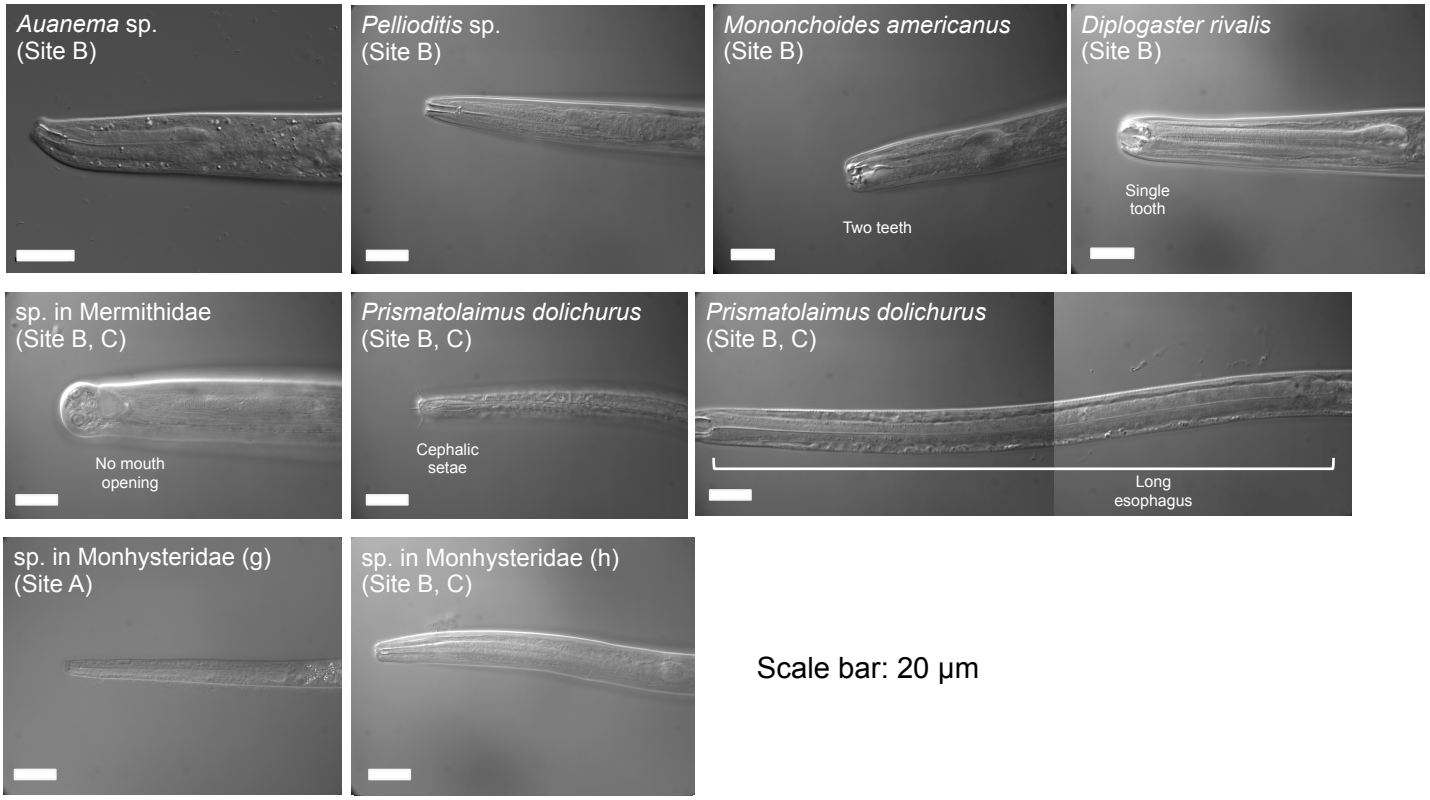


D

Year	Site A			Site B			Site C		
	dry zone	tide zone	lake	dry zone	tide zone	lake	dry zone	tide zone	lake
2016	0/1	1/5	1/3	2/3	9/14	0/1	1/1	0/6	NA
2017	0/1	2/6	3/8	NA	1/3	0/9	6/14	3/4	4/11
Total	0%	27%	36%	67%	59%	0%	47%	30%	36%

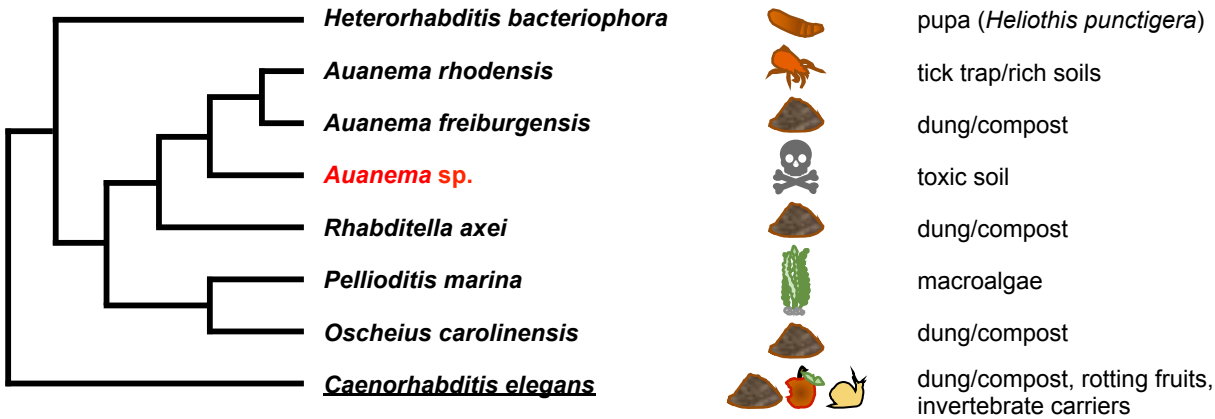
Figure S1. Sample summaries. Related to Figure 1
(A) Pictures of Site A, Site B, and Site C. **(B)** Plot of locations at Site A where samples were collected, relative to the shore (x-axis) and surface (y-axis). Green, land; blue, lake water; brown, sedimentary soil. Circles, samples collected in 2016; squares, 2017. Pink, samples in which nematodes were found. **(C)** Plot of the salinity and pH of measured samples. Each dot represents the measurements from one single sample. Color corresponds to the site where the sample was collected. **(D)** Presence of nematodes at each site. Proportions indicate the number of samples that contained nematodes over the total number of collected samples. Beige, locations where nematodes were isolated in 2016; grey, 2017. NA, non-applicable.

A



Scale bar: 20 µm

B



C

Species	Sequence identity			
	LSU	Closest relative	SSU	Closest relative
Auanema sp. (a)	89%	*A. rhodensis*	96%	*A. rhodensis*
Pellioditis sp. (b)	88%	*Pellioditis* sp.	95%	*Pellioditis* sp.
Mononchoides americanus (c)	90%	*M.* sp.	98%	*M. americanus*
Diplogaster rivalis (d)	92%	*Butlerius* sp.	99%	*D. rivalis*
species in Mermithidae (e)	85%	*Romanomermis culicivorax*	93%	*Mermis nigrescens*
Prismatolaimus dolichurus (f)	99%	*P. dolichurus*	NA	
species in Monhysteridae (g)	NA		92%	*Monhysteridae* sp.
species in Monhysteridae (h)	NA		96%	*Monhysteridae* sp.
species g to species h	NA	--	97%	--

Figure S2. Classification of Mono Lake nematodes. Related to Figure 2
(A) Morphology of species a-h under high magnification. **(B)** Simplified phylogenetic tree showing the relationships of *Auanema* sp. (red) and selected Rhabditina, based on SSU sequences. **(C)** Sequence identity of each isolate, compared to its closest relative. Red, known species.

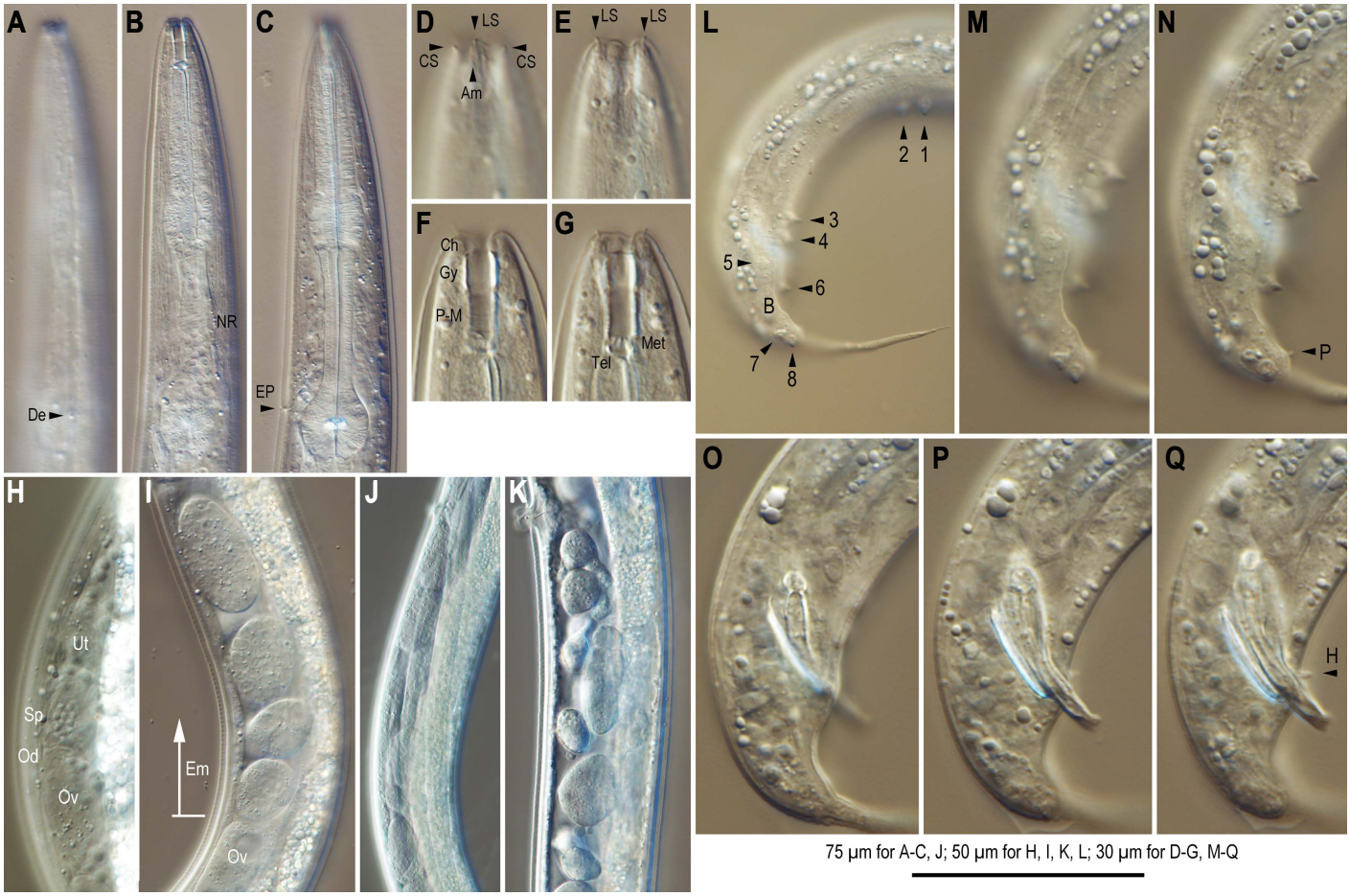
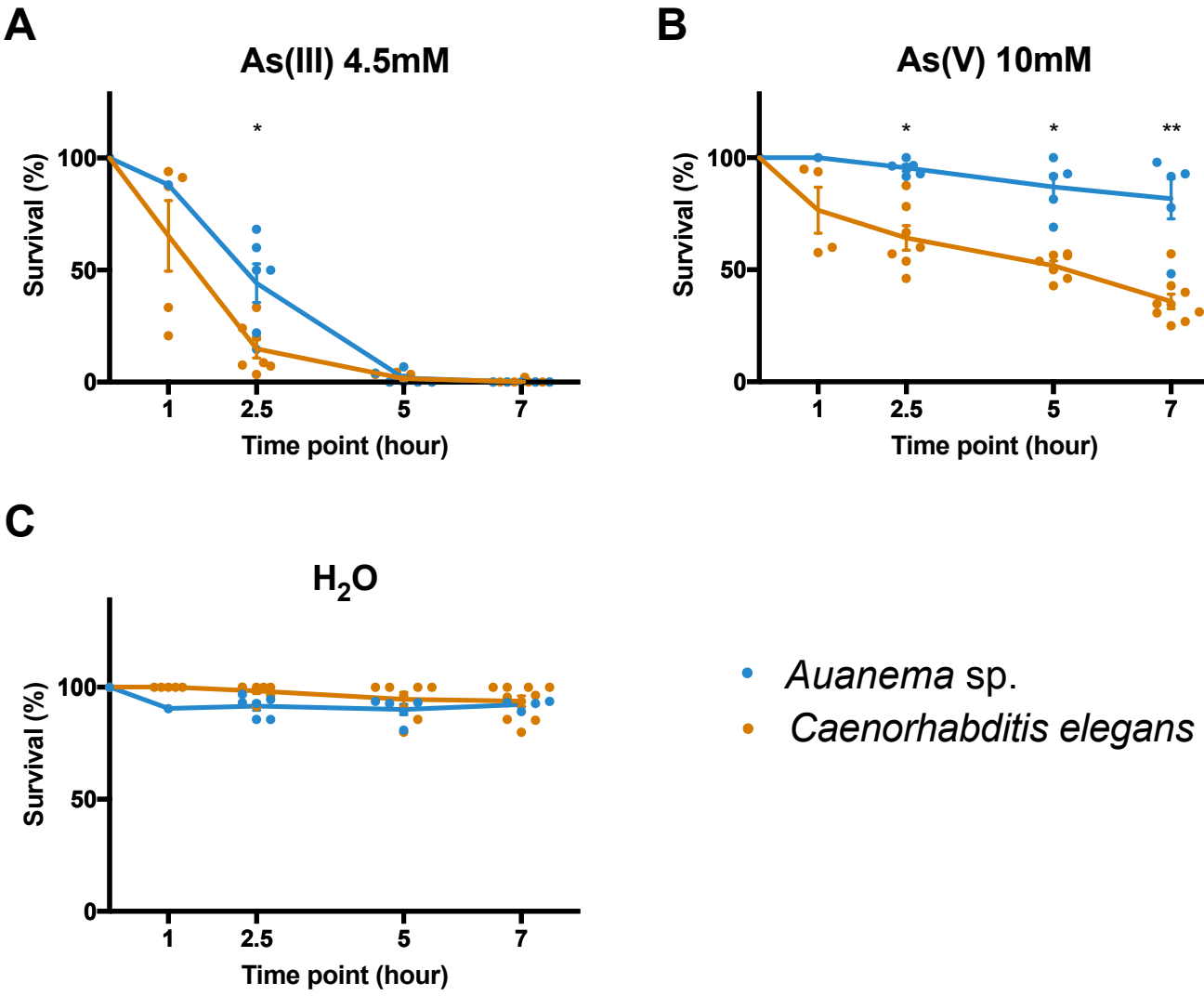


Figure S3. Typological and biological characters of *Auanema* sp. Related to Figure 3
(A-G) Anterior region of *Auanema* sp. in left lateral view. A-C: Anterior region in different focal planes; D-G: Stomatal region in different focal planes. Abbreviations are as follows, De: deirid; NR: nerve ring; EP: excretory pore opening; CS: Cephalic sensilla; LS: labial sensilla; Am: amphid; Ch: cheilostom; Gy: gymnostom; P-M: pro- and mesostegostom; Met: metastegostom; Tel: telostegostom. **(H-K)** Hermaphrodite and female gonads of *Auanema* sp. H: Young hermaphrodite possessing sperm in its spermatheca; I: Young hermaphrodite with developing embryos; J: Mature hermaphrodite with juveniles and developed embryos; K: Unmated mature female with undeveloped oocytes. Abbreviations are as follows, Ut: uterus; Sp: sperm; Od: oviduct; Ov: ovary; Em: embryo. **(L-Q)** *Auanema* sp. male tail in right lateral view. L: Whole tail region; M-Q: Close-up of posterior part in different focal planes. Abbreviations are as follows, numbers: genital papillae; B: bursa; P: phasmid; H: ventral single papilla (hook).



D

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C. elegans (Bristol)        VQVKEWYVKEGDTISQFDKVCEVQSDKAAVTISCRYDGIVKKLYHEVDGMARVGQALIDV  104
C. elegans (Hawaii)         VQVKEWYVKEGDTISQFDKVCEVQSDKAAVTISSRYDGIVKKLYHEVDGMARVGQALIDV  104
A. freiburgensis (APS7)     VQVKEWYVKEGDTVSQFDNLCDVQSDKAAVTITSRYDGVIRKLHHNIDDLARVGLPLVDI  105
A. rhodensis (APS4)         VQVKEWYVKEGDTVSQFDNLCDVQSDKAAVTITSRYDGVVRKLHHKIDDLARVGLPLVDI  130
A. sp. (PS8402)             VQVKEWYVKEGDNISQFDNLCDVQSDKAAVTITSRYDGVVRKLYHKIDDLARVGQPLVDI
                            ************.:****::*:**********:.****:::**:*:::*.:****  *:*:

C. elegans (Bristol)        EIEGNVEEPEQPKKEAASSSPEAPK-------SSAPKAPESAHSEGKVLATPAVRRIAIE  157
C. elegans (Hawaii)         EIEGNVEEPEQPKKEAASSSPEAPK-------SSAPKAPESAHSEGKVLATPAVRRIAIE  157
A. freiburgensis (APS7)     EIEGEGEEESSNDSKKTE--T-TRETSTSSPSATTQNASEFQGTHGKVLATPAVRRIAME  162
A. rhodensis (APS4)         EIEGEEETTSEAEKPA---ETGASNATSTSSTSSVQSSNDFHAVNGKVLATPAVRRIAME  187
A. sp. (Mono Lake)          EVDDEVAEDSKESKESVSSSVTSPEANATPSPSSVQSSSDFHSTNGKVLATPAVRRIAME
                            *::.:    .. ..       : :       ::. .: :    .**************:*

C. elegans (Bristol)        NKIKLAEVRGTGKDGRVLKEDVLKFL         183
C. elegans (Hawaii)         NKIKLAEVRGTGKDGRVLKEDVLKFL         183
A. freiburgensis (APS7)     NKVDLSAVPATGKEGRVLKEDILKFL         188
A. rhodensis (APS4)         NKVKLSDVPSTGKDGRVLKEDILKFL         213
A. sp. (PS8402)             NKVKLQDVPASGKDGRVLKEDILKSS
                            **:.*  * .:**:*******:**
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Figure S4. *Auanema* sp. is arsenic resistant. Related to Figure 4
(A-C) Survival of *Auanema* sp. (blue) and *C. elegans* (orange) adults in arsenic solutions and water-only control at 1, 2.5, 5, and 7 hour time points. Jitter is added for clarity. Points represent individual populations (average 32 animals), bars denote the standard error of the mean. Statistics: non-parametric permutation test at each time point. "*" $q < 0.05$, "**" $q < 0.01$. **(D)** Alignment of DBT-1 protein sequence surrounding the Cys/Ser variant position (yellow).

Sample number	Location	From shore (cm)	Under ground (cm)	Water depth (cm)	Weight of sample (g)	pH	Salinity (ppt)	Nematodes present	Number of nematodes / species
A1	tide zone	0	0	0	368	ND	ND	no	
A2	tide zone	-150	0	0	295	ND	ND	no	
A3	tide zone	0	10	0	350	ND	ND	no	
A4	tide zone	0	0	0	499	9.477	14.342	**yes**	1 / 1
A5	tide zone	-100	0	0	348	ND	ND	no	
A6	dry zone	-900	23	0	216	ND	ND	no	
A7	in-lake	1,800	10	30	367	ND	ND	no	
A8	in-lake	7,000	6	50	680	9.624	10.26	**yes**	~5 / 1
A9	in-lake	2,100	9	30	186	ND	ND	**yes**	ND / 1
A100	tide zone	5	10	0	18.1	10.21	16.5	no	
A101	tide zone	5	5	0	20.7	10.08	16.8	**yes**	1 / 1
A102	dry zone	-300	5	0	41.8	10.12	17.02	no	
A103	tide zone	50	0	5	31.9	10.15	14.3	no	
A104	tide zone	0	5	0	33.1	10.02	18.6	no	
A105	in-lake	30	0	5	26.7	10.01	11.7	no	
A106	tide zone	-150	0	0	27.5	10.03	17.2	no	
A107	tide zone	-100	0	5	21.6	10.03	10.8	**yes**	4 / ND
A108	in-lake	100	5	10	250	10.28	13	**yes**	33 / ND
A109	in-lake	1,000	5	20	241	10.33	9.65	**yes**	1 / 1
A110	in-lake	5,000	5	20	20.8	10.24	8.93	no	
A111	in-lake	10,000	5	30	26.8	10.25	8.58	**yes**	4 / ND
A112	in-lake	20,000	5	50	26	9.94	7.02	no	
A113	in-lake	30,000	5	70	22.8	10.15	9.17	no	
A114	in-lake	100	5	5	26.4	10.26	9.65	no	
B1	tide zone	0	0	0	ND	ND	ND	no	
B2	tide zone	0	0	0	143	ND	ND	no	
B3	in-lake	300	0	10	143	ND	ND	no	
B4	tide zone	0	8	0	318	ND	ND	no	
B5	tide zone	20	10	0	157	ND	ND	**yes**	2 / 1
B6	tide zone	10	10	0	211	ND	ND	**yes**	1 / 1
B7	tide zone	0	5	0	175	9.3925	0.581	**yes**	~15 / 2
B8	tide zone	-100	5	0	231	ND	ND	**yes**	20 / ≥2
B9	tide zone	-100	5	0	162	5.967[a]	0.779	**yes**	~50 / 3
B10	tide zone	0	0	0	248	ND	ND	**yes**	1 / 1
B12	tide zone	0	0	0	330	ND	ND	**yes**	1 / 1
B13	tide zone	-75	8	0	113	ND	ND	no	
B14	tide zone	-75	0	0	311	8.394	0.586	**yes**	~200 / ≥3
B15	tide zone	0	0	0	187	ND	ND	no	
B16	dry zone	-300	6.5	0	222	ND	ND	**yes**	2 / ND
B19	dry zone	-300	5	0	372	ND	ND	no	
B20	dry zone	0	0	0	192	7.815	1.022	**yes**	~20 / 2
B21	tide zone	-120	4	0	133	ND	ND	**yes**	1 / 1
B100	in-lake	30	10	0	32.7	9.42	1.19	no	
B101	tide zone	0	10	0	22	8.83	0.725	no	
B102	in-lake	300	10	0	22.9	8.27	0.166	no	
B103	in-lake	50	10	30	20.3	9.58	2.74	no	
B104	in-lake	10	10	10	24.1	9.97	2.7	no	
B106	in-lake	10	10	10	26.7	8.76	1.16	no	
B107	in-lake	30	10	10	16.7	9.26	1.692	no	
B108	tide zone	-100	10	0	25.5	8.29	0.214	**yes**	40 / 1
B111	tide zone	0	10	0	30.3	10.02	2.29	no	
B112	in-lake	100	10	40	25.5	10.2	5.08	no	
B113	in-lake	300	10	50	31.4	10.26	4.65	no	
B114	in-lake	1,000	10	100	30.9	10.2	5.18	no	
C1	tide zone	0	5	0	168	ND	ND	no	
C2	tide zone	0	0	0	204	ND	ND	no	
C3	tide zone	-30	0	0	245	ND	ND	no	
C4	tide zone	0	4	0	62	ND	ND	no	
C6	tide zone	0	5	0	53	ND	ND	no	
C7	dry zone	-200	5	0	221	10.2145	5.87	**yes**	3 / 2
C8	tide zone	0	3	0	85	9.175	22.361[a]	no	
C100	in-lake	300	0	0	20.8	10.06	4.17	**yes**	1 / 1
C101	tide zone	5	0	0	26.5	9.89	4.76	**yes**	2 / ND
C102	dry zone	-20	0	0	27.9	10.02	7.17	**yes**	8 / 1
C103	in-lake	10	20	0	26.4	10.02	5.13	no	
C104	dry zone	-1,000	0	0	19.7	10.03	12.34	**yes**	52 / ND
C105	in-lake	20	20	20	23	9.92	3.5	no	
C106	tide zone	0	0	0	21.6	9.15	5.72	**yes**	1 / 1
C108	in-lake	100	0	20	19.2	9.94	5.08	no	
C109	in-lake	100	10	0	10.6	9.96	7.26	no	
C110	in-lake	300	10	40	9.2	9.9	7.06	**yes**	2 / ND
C111	in-lake	1,000	0	50	12.5	10.09	5.8	**yes**	2 / ND
C112	in-lake	3,000	10	50	37.2	9.84	7.44	**yes**	1 / 1
C113	in-lake	5,000	0	50	25	9.96	6.26	no	
C114	in-lake	7,000	10	70	18.1	9.84	8	no	
C115	in-lake	10,000	10	100	18.3	9.76	9.76	no	
C130	dry zone	-300	5	0	ND	ND	ND	**yes**	~400 / 1
C131	dry zone	-500	5	0	ND	ND	ND	**yes**	131 / 2
C132	tide zone	-30	5	0	ND	ND	ND	no	
C133	dry zone	-1,000	5	0	ND	ND	ND	**yes**	10 / 1
C134	dry zone	-1,000	15	0	ND	ND	ND	no	
C135	dry zone	-1,000	28	0	ND	ND	ND	no	
C136	dry zone	-500	15	0	ND	ND	ND	**yes**	1 / 1
C137	dry zone	-500	48	0	ND	ND	ND	no	
C138	dry zone	-50	5	0	ND	ND	ND	no	
C139	dry zone	-50	48	0	ND	ND	ND	no	
C140	dry zone	-50	28	0	ND	ND	ND	no	
C141	dry zone	-50	15	0	ND	ND	ND	no	
C142	dry zone	-10,000	2	0	ND	ND	ND	no	
C143	tide zone	-30	5	10	ND	ND	ND	**yes**	1 / 1

Table S1. Soil sample records. Related to Figure 1

Sample numbers indicate the sampling site (A, B, or C) and sampling year (2016 samples start from 1, 2017 samples start from 100). The sign indicates the direction of the sampling: into the lake (positive) or away from the lake (negative). Footnote: a, outliers, excluded from further analysis. ND: not determined.

Species		Site isolated	Date isolated	Isolated alongside	Total number of nematodes from site (all species)	Lifestyle	Clade	Morphology
Auanema sp. (**a**)	PS8402	Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B14**), -75 cm from shore, 0 cm underground, pH 8.394, salinity 0.586 ppt.	August 2, 2016.	Two unidentified nematode species.	~200	Microbe-feeding.	V	Viviparous reproductive mode. Hermaphrodites, females, males observed. Grinder in terminal bulb of the pharynx. Additional descriptions in Suppl. Text.
	PS8403	Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B8**), -100 cm from shore, 5 cm underground.	August 2, 2016.	*Diplogaster rivalis* & species in Monhysteridae.	20			
Pellioditis sp. (**b**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B9**), -100 cm from shore, 5 cm underground, pH 5.967, salinity 0.779 ppt.	August 2, 2016.	*Mononchoides americanus* & species in Mermithidae.	~50	Microbe-feeding.	V	Intestine is golden-colored. Grinder in terminal bulb of the pharynx. Tail is long and tapered.
		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), dry zone sediments from close to weeds (**B20**), pH 7.815, salinity 1.022 ppt.	August 2, 2016.	*Mononchoides americanus*.	~20			Males with a brush-like spicule observed.
Mononchoides americanus (**c**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B7**), 0 cm from shore, 5 cm underground, pH 9.393, salinity 0.581 ppt.	August 2, 2016.	*Prismatolaimus dolichurus*.	~15	Predatory.	V	Two teeth. Hermaphrodites/females observed. See Chitwood and Chitwood (1937), Calaway and Tarjan (1973).
		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B9**), -100 cm from shore, 5 cm underground, pH 5.967, salinity 0.779 ppt.	August 2, 2016.	*Pellioditis* sp. & species in Mermithidae.	~50			
		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), dry zone sediments from close to weeds (**B20**), pH 7.815, salinity 1.022 ppt.	August 2, 2016.	*Pellioditis* sp.	~20			
Diplogaster rivalis (**d**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B8**), -100 cm from shore, 5 cm underground.	August 2, 2016.	*Auanema* sp. & species in Monhysteridae.	20	Predatory.	V	Single tooth. Hermaphrodites/females observed. Two-armed gonad.
species in Mermithidae (**e**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B9**), -100 cm from shore, 5 cm underground, pH 5.967, salinity 0.779 ppt.	August 2, 2016.	*Pellioditis* sp. & *Mononchoides americanus*.	~50	Parasitic.	I	No mouth opening observed. Long compared to other Mono Lake nematodes: approximately 4 mm in length. Tail rounded and nubbed.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C131**), -500 cm from shore, 5 cm underground.	July 15, 2017.	One unidentified nematode species.	131			Hermaphrodites/females and males observed.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C133**), -1000 cm from shore, 5 cm underground.	July 15, 2017.	NA.	10			
Prismatolaimus dolichurus (**f**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B7**), 0 cm from shore, 5 cm underground, pH 9.393, salinity 0.581 ppt.	August 2, 2016.	*Mononchoides americanus*.	~15	Predatory.	II	Small teeth-like structures. Prominent cephalic setae. Long esophagus, 2/5 the length of the body.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C130**), -300 cm from shore, 5 cm underground.	July 15, 2017.	NA.	~400			Original description de Man, J.G. (1880).
species in Monhysteridae (**g**)		Pristine Beach, (38° 3' 27.91" N, 119° 1' 50.66" W), in-lake sediments (**A9**), 2100 cm from shore, 9 cm underground, 30 cm water depth.	August 2, 2016.	NA.	ND.	Microbe-feeding.	(II, III)	Grinder observed. Adults with single egg in uterus observed.
species in Monhysteridae (**h**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B8**), -100 cm from shore, 5 cm underground.	August 2, 2016.	*Auanema* sp. & *Diplogaster rivalis*.	20	Microbe-feeding.	(II, III)	Grinder in terminal bulb of pharynx. Adults with single egg in uterus observed.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C7**), -200 cm from shore, 5 cm underground, pH 10.214, salinity 5.87 ppt.	August 3, 2016.	One unidentified nematode species.	3			

Table S2. Species records. Related to Figures 1 and 2

Lifestyles are predicted by morphology and phylogeny, or observed (*Auanema* sp.) The Clade I-V system by Blaxter is used here.

Supplemental References

S1. Kanzaki, N., Tanaka, S.E., Fitza, K., Kosaka, H., Slippers, B., Kimura, K., Tsuchiya, S., and Tabata, M. (2016). *Deladenus nitobei* n. sp. (Tylenchomorpha: Allantonematidae) isolated from *Sirex nitobei* (Hymenoptera: Siricidae) from Aomori, Japan, a new member of the siricidicola superspecies. Nematology 18: 1199-1217. DOI: 10.1163/15685411-00003025

S2. Herrmann, M., Ragsdale, E.J., Kanzaki, N., and Sommer, R. (2013). *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. (Nematoda: Diplogastridae): viviparous new species with precocious gonad development. Nematology 15: 1001-1020. DOI: 10.1163/15685411-00002738

S3. Kanzaki, N., Giblin-Davis, R.M., Gonzalez, R., Wood, L.A., and Kaufman, P.E. (2017). *Sudhausia floridensis* n. sp. (Nematoda: Diplogastridae) isolated from *Onthophagus tuberculifrons* (Coleoptera: Scarabaeidae) from Florida, USA. Nematology 19: 575-586. DOI: 10.1163/15685411-00003071

S4. Kanzaki, N., Kiontke, K., Tanaka, R., Hirooka, Y., Schwarz, A., Müller-Reichert, T., Chaudhuri, J., and Pires da Silva, A. (2017). Description of two three-gendered nematode species in the new genus *Auanema* (Rhabditina) that are models for reproductive mode evolution. Scientific Reports 7: 11135. DOI: 10.1038/s41598-017-09871-1